

## BRIEF COMMUNICATIONS

Engineering *Escherichia coli* to see light

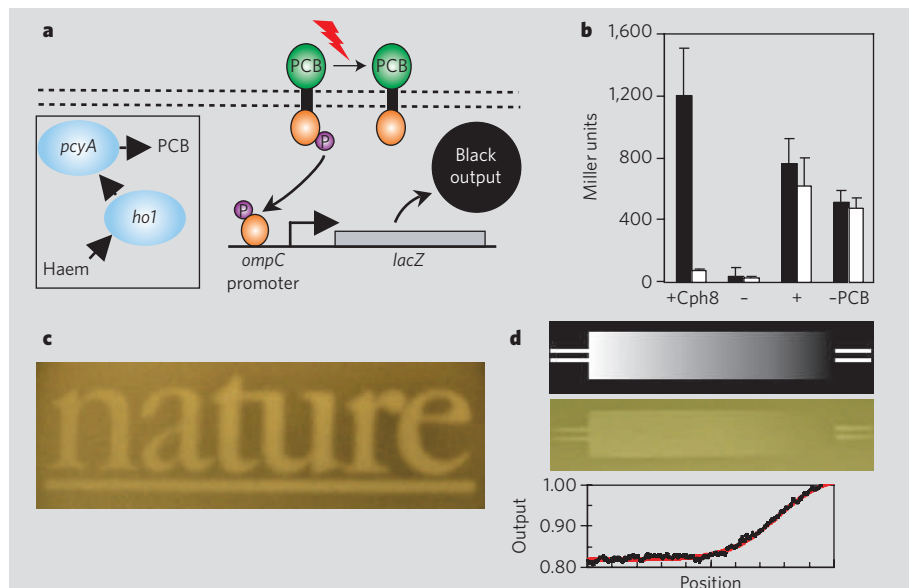
These smart bacteria 'photograph' a light pattern as a high-definition chemical image.

We have designed a bacterial system that is switched between different states by red light. The system consists of a synthetic sensor kinase that allows a lawn of bacteria to function as a biological film, such that the projection of a pattern of light on to the bacteria produces a high-definition (about 100 megapixels per square inch), two-dimensional chemical image. This spatial control of bacterial gene expression could be used to 'print' complex biological materials, for example, and to investigate signalling pathways through precise spatial and temporal control of their phosphorylation steps.

Plants and some bacteria use a class of protein photoreceptors known as phytochromes to control phototaxis, photosynthesis and the production of protective pigments<sup>1–3</sup>. Photoreceptors are not found in enterobacteria, such as *Escherichia coli*, so we created a light sensor that functions in *E. coli* by engineering a chimaera that uses a phytochrome from a cyanobacterium.

A phytochrome is a two-component system that consists of a membrane-bound, extra-cellular sensor that responds to light and an intracellular response-regulator<sup>1</sup>. The response-regulators of most phytochromes do not have DNA-binding domains and do not directly regulate gene expression, so we fused a cyanobacterial photoreceptor to an *E. coli* intracellular histidine kinase domain (Fig. 1a, and see supplementary information). This design was based on the well studied *E. coli* EnvZ–OmpR two-component system, which normally regulates porin expression in response to osmotic shock<sup>4</sup>. The EnvZ histidine kinase domain has been used for the construction of functional chimaeras<sup>5,6</sup>, and a plant phytochrome has previously been used to construct a two-hybrid gene expression system in yeast<sup>7</sup>.

To create the chimaera, we aligned members of the phytochrome family with EnvZ and identified potential functional crossover points between the *Synechocystis* phytochrome Cph1 and EnvZ. (For methods, see supplementary information.) The length and composition of the peptide that links a photoreceptor to its response-regulator can affect signal transduction<sup>5,6</sup>, and we therefore constructed a series of chimaeras with variable linker lengths. The variants were transformed into a  $\Delta$ EnvZ *E. coli* strain containing a chromosomal fusion between the OmpR-dependent *ompC* promoter and the *lacZ* reporter<sup>4</sup>, which



**Figure 1 | Light imaging by engineered *Escherichia coli*.** **a**, The chimaeric light receptor Cph8 contains the photoreceptor from Cph1 (green) and the histidine kinase and response-regulator from EnvZ–OmpR (orange); inset, conversion of haem to phycocyanobilin (PCB), which forms part of the photoreceptor. Red light drives the sensor to a state in which autophosphorylation is inhibited (right), turning off gene expression. For details of genes, see text. **b**, Miller assay showing that Cph8 is active in the dark (black bars) in the presence of PCB and inactive in the light (white bars). There is no light-dependent activity in the absence of Cph8 (–) and there is constitutive activity when only the histidine kinase domain of EnvZ is expressed (+), or when the PCB metabolic pathway is not included (– PCB). **c**, When an image is projected on to a bacterial lawn, the *lacZ* reporter is expressed only in the dark regions. **d**, Transfer function of the circuit. As the intensity of the light is increased by using a light gradient projected from a 35-mm slide, the circuit output gives a graded response.

enzymatically produces a black compound.

The part of the photoreceptor that responds to light, phycocyanobilin, is not naturally produced in *E. coli*. We therefore introduced two phycocyanobilin-biosynthesis genes (*ho1* and *pcyA*) from *Synechocystis* that convert haem into phycocyanobilin<sup>8</sup> (parts BBa\_I15008, BBa\_I15009; MIT Registry of Standard Biological Parts) (Fig. 1a, inset). Individual Cph1–EnvZ chimaeras were then activated at 37 °C for 4 h with broad-spectrum light and assayed for expression of the *lacZ* reporter. The chimaera Cph8 (BBa\_I15010) produced a particularly strong response to light (Fig. 1b).

For bacterial photography, we grew a lawn of bacteria on agar. The *lacZ* reporter was visualized by addition of S-gal (3,4-cyclohexenoesculetin- $\beta$ -D-galactopyranoside): LacZ catalyses the formation of a stable, insoluble, black precipitate from S-gal. Light repressed gene expression in the bacteria, giving a high-contrast replica of the applied image on

the biological film, in which light regions appeared light and dark regions were dark (Fig. 1c, and see supplementary information). The *lacZ* activity showed a graded response to increasing light intensity that was minimal in the brightest light (Fig. 1d).

Our creation of a novel genetic circuit with an image-processing function demonstrates the power and accessibility of the tool sets and methods available in the nascent field of synthetic biology. The principle of programmed light regulation should enable gene expression to be spatially and temporally controlled in individual cells and in populations, leading to potential application in bacterial microlithography, manufacture of biological material composites and the study of multicellular signalling networks.

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## INSECT COMMUNICATION

# 'No entry' signal in ant foraging

Forager ants lay attractive trail pheromones to guide nestmates to food<sup>1,2</sup>, but the effectiveness of foraging networks might be improved if pheromones could also be used to repel foragers from unrewarding routes<sup>3,4</sup>. Here we present empirical evidence for such a negative trail pheromone, deployed by Pharaoh's ants (*Monomorium pharaonis*) as a 'no entry' signal to mark an unrewarding foraging path. This finding constitutes another example of the sophisticated control mechanisms used in self-organized ant colonies.

To investigate whether foragers lay a negative signal on the unrewarding branch of a trail bifurcation, we removed paper substrate from immediately after the fork on the unrewarding branch (the other branch led to a sucrose feeder) after it had been used by a trail-laying colony of ants. This paper substrate was transferred to the entrance of one branch of a similar set-up, in which both branches had previously led to sucrose and had been used by a second colony of ants. The other branch of the second set-up received a neutral control paper substrate (for details, see supplementary information). Foragers walking from the nest could choose either of the test branches or make a U-turn.

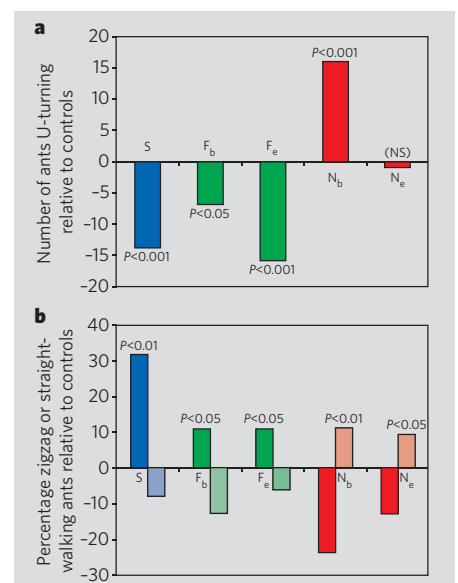
We found that 69% continued to walk away from the nest and make a branch choice. Of these, most (71%) chose the branch with the control substrate ( $\chi^2=22.1$ , d.f.=1,  $n=137$ ,  $P<0.001$ ); the remainder U-turned towards the nest on reaching the trail bifurcation. U-turns were more than four times as likely if the ant had contacted the unrewarding-branch substrate (55%) as opposed to the neutral-control substrate (13%) ( $\chi^2=40.9$ , d.f.=1,  $n=200$ ,  $P<0.0001$ ). Neither substrate came from a previously rewarding trail, so this result cannot be attributed to differences in positive-trail pheromone concentrations.

We next investigated the negative signal's location by taking substrate from five locations on a bifurcating trail that had one rewarding

and one unrewarding branch. These sections, along with neutral controls, were tested on unbranched foraging trails (see supplementary information) by noting whether individual foragers walking over them did a U-turn. Compared with ants on the control substrate, almost twice as many ants U-turned when walking on substrate from the unrewarding branch near the bifurcation ( $N_b$ ) (19% and 34%, respectively;  $P<0.001$ ) (Fig. 1a). However, U-turns were as frequent on substrate from the unrewarding branch end ( $N_e$ ) (27%) as on the control (27%) (NS) (Fig. 1a). Ants U-turned less often on sections from the rewarding trail (stem S, 12%; feeder branch close to the bifurcation  $F_b$ , 12%; and feeder-branch end  $F_e$ , 13%). These values are significantly lower than those for the relevant control (S,  $P<0.001$ ;  $F_b$ ,  $P<0.05$ ;  $F_e$ ,  $P<0.001$ ) (Fig. 1a).

In the same experiment, we also determined whether foragers could detect the negative signal before reaching the substrate on which it had been laid, using walking behaviour (zigzagging versus walking straight) as a bio-assay. Our results show that significantly more ants zigzagged when approaching substrate from an unrewarding branch just after the bifurcation ( $P<0.01$ ) or at the branch end ( $P<0.05$ ) than did controls (Fig. 1b). Conversely, significantly fewer zigzagged when approaching substrate leading to the feeder (S,  $P<0.01$ ;  $F_b$ ,  $P<0.05$ ;  $F_e$ ,  $P<0.05$ ) (Fig. 1b).

Our results show that Pharaoh's ants use a sophisticated trail system with a negative, repellent pheromone to mark unrewarding branches. The signal is concentrated at decision points — trail bifurcations<sup>5</sup>. As it is volatile, it provides advance warning — like human road signs situated before junctions. Across a trail network, the pheromone could help direct foragers to food by closing off unrewarding sections. Exactly how negative pheromones enhance foraging efficiency in trail networks is not known, but they might complement attractive trail pheromones<sup>6,7</sup>



**Figure 1 | Identifying the location of the negative pheromone.** The ants' response is monitored by their walking behaviour, with U-turning or zigzagging on unbranched trails indicating detection. Test sections: S, 1 mm before bifurcation;  $F_b$  and  $N_b$ , 3 mm after bifurcation on feeder and non-feeder branches, respectively;  $F_e$  and  $N_e$ , 60 mm from bifurcation at the ends of feeder and non-feeder branches, respectively. (For details and chi-squared tests, see supplementary information.) **a**, Number of ants that U-turned while walking on different test sections, relative to controls. **b**, Percentage of straight-walking (left bars) or zigzagging (right bars) ants, relative to controls.

used by Pharaoh's ants in trail choice, or they could prevent strong positive feedback by attractive pheromones from locking the system into suboptimal solutions<sup>1,8</sup>.

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